THERMAL RESISTANCE, DESICCATION, AND AEROSOLIZATION

The thermal resistance of a microorganism is influenced by all environmental conditions it encounters in its life history. Spores are much more resistant than vegetative cells. Some of the better known factors which play important roles in the apparent heat resistance of a culture include the composition of the sporulation medium, temperature during sporulation, the species and particular strain, the age of the spores, degree of hydration, the spore carrier, the gaseous atmosphere during heat resistance studies in the dry state, the composition of the heating menstruum surrounding spores heated in liquid, the requirement for activation by heat or chemicals to trigger germination and break dormancy, the composition of the culture medium in viability studies, and the incubation temperature. Several of these determinants of apparent heat resistance will be considered briefly. Sensitization of organisms to heat by ionizing and ultraviolet radiations is treated elsewhere in this report.

Thermal Resistance in Dry and Moist Environments.

Bruch (168) reviewed the biological and physical factors in dry heat sterilization. He cited the enhancing action of vacuum against organisms exposed on surfaces, differences in the order of

sensitivity of spores in several hot gaseous atmospheres, and the influence of moisture in the hot gases on spore resistance. He observed that solids are difficult to sterilize by dry heat. The presence of plastics in space probes will make sterilization difficult.

Jaffe (169) noted that many spacecraft components are damaged in 24 hours at 135 C. Heat sterilization might be useful in flight, but it would be difficult to heat a spacecraft uniformly and avoid interference with instrument calibration.

Bělehrádek (62) compiled a table of temperature coefficients of the rate of injury by heat for a number of spores and bacteria. Over the temperature range 120 - 135 C, dry Bacillus anthracis spores had a Q_{10} value of 2.9.

Koesterer and Bruch (69) compared the resistance of dry spores of Bacillus subtilis var. niger and B. stearothermophilus strain 1518 to destruction by moist and dry heat. Sterilization of 1-10 X 10^5 spores was accomplished in less than 5 min for B. subtilis var. niger at 110 C, and in 20 min at 121 C for B. stearothermophilus. B. subtilis var. niger spores were 3.5 to 5 times more resistant to dry heat than B. stearothermophilus spores. The times required to kill 90 percent of the spores at 121 C were 47.5 and 14.3 min, and 1.6 and 0.3 min at 160 C respectively, for B. subtilis var. niger and B. stearothermophilus.

Bruch, Koesterer and Bruch (70) considered the dry heat resistance of spores on several carriers. This research was aimed at development of dry heat cycles for sterilizing spacecraft components. In experiments at 125 C, the presence of soil, sand, and vermiculite increased the apparent heat resistance well beyond the resistance of spores on paper strips or in test tubes.

Pheil, Nicholas, and Pflug (77) showed that the nature of the dry gas surrounding cells during heating influences the destruction rate of Bacillus subtilis spores. Helium and nitrogen were less lethal than oxygen, air and carbon dioxide.

Wende and Burdon (66) investigated the dry heat sterilization of bonemeal contaminated with Bacillus anthracis spores. Samples weighing 25 g were sterilized after 60 min at 160 C while 40 min sufficed for 10 g samples. Morphological and toxigenic variants increased with the degree of heat exposure.

Zampach (64) sterilized plastic bands of a microbial air sampling device with dry heat at 140 C in 3 hr. A revolving drum was sterilized by means of an antiseptic such as mercury phenylborate.

Davis, et al. (71, 81, 187) observed that spores varied in resistance to moderate temperatures in ultrahigh vacuum. Viable Aspergillus niger spores were recovered after 4-5 days in ultrahigh vacuum at 107 C, but none were viable at 120 C. B. subtilis var.

niger spores did not survive 90 C in vacuum, but significant numbers were recovered in experiments conducted for 5 and 7 days at 90 C and atmospheric pressure.

Henderson and Dinning (73) obtained 0.1-1 percent survival of dry Aspergillus niger spores after 3 hr at 100 C. When the spores were suspended in water, only 0.00001 percent were recovered after 1 hr at 100 C. Spores in deuterium oxide were 1,000 to 10,000 times more resistant than spores in water.

An exceptionally heat resistant Bacillus subtilis was isolated by Iyer and Bhat (63) from Bombay air. Survivors were observed after 60 min at 120 C in aqueous suspensions containing 10^5 spores.

Sporulation Environment and Thermal Resistance.

The thermal resistance of Bacillus coagulans spores was found by Lechowich and Ordal (67) to increase with increased sporulation temperature. The cation contents of these spore crops were similar, but the dipicolinic acid content decreased as the sporulation temperature increased.

Tallentire and Chiori (109) were able to double heat resistance in Bacillus megaterium spores produced in an Mn-Ca-Mg medium instead of an Mg-Fe medium.

Spore Activation and Determination of Viability.

The environmental and nutritional requirements for spore germination are poorly defined. Heating spores at temperatures

usually considered lethal for vegetative cells is often necessary to achieve maximal colony counts under 'normal' incubation conditions. These 'heat shock' temperature-time relationships are quite arbitrary, and there is no assurance of quantitative recovery of all viable spores. It is possible that stresses imposed on an organism undergoing sublethal heating or sublethal irradiation alter the normal metabolic and growth patterns so that although the spore is viable, the environment is not conducive for proliferation.

An organism exposed to a combined treatment with two agents might be more refractory than under a single environmental stress. Other sections of the report illustrate the reversibility of apparent lethality by agents seemingly unrelated to one or more damaging physical or chemical sterilant. Several citations serve to illustrate the problems encountered in assessing microbial survival.

Segner (74) obtained a tenfold increase in viable count when Bacillus stearothermophilus spores were heated to 120 C and cooled, compared with counts after heat activation at 100 C.

Graikoski and Kempe (86) found that activation of dormant Clostridium botulinum Type E spores heated in neutral buffer occurred during 40-60 min heating at 70 and 75 C although significant

numbers of spores were killed in 20 min. Survivors were obtained after heating for 60 min at 90 C.

Thermal resistance of spores heated in suspension can depend on the buffer composition and pH (80).

The composition of culture media for heat-treated bacteria can influence the apparent survival. Low Co^{++} concentrations increase survival of sublethally heated nonsporulating bacteria five-to-tenfold, while Cu^{++} is inhibitory (82).

The highest growth temperatures confirmed for microorganisms are 72-75 C (85).

A mesophile resembling Bacillus megaterium was able to grow at 65 C in association with a thermophile resembling B. stearothermophilus (83). Johnson and Chan (78) found that Arthrobacter globiformis, a soil organism that grows well at 25 C, would grow at 30 and 37 C only in the presence of another organism which could grow at these temperatures. It would be interesting to determine whether presumably sterile surfaces do indeed harbor viable organisms which require commensal association to grow at usual incubation temperatures.

The longevity of anaerobic sporeformers was reported by Haynes and Rhodes (56) to exceed 13 years in lyophil preparations and 20 years in soil.

Microbial Aerosols.

Levin and Cabelli (53) reported that germination of Bacillus subtilis var. niger spores occurred in the processes of aerosolization and collection in liquid impingers. Germination was dependent on oxygen and independent of exogenous nutrients. The spores collected had increased heat susceptibility.

Cabelli (51) and Hayes and Cabelli (58) attributed loss in Pasteurella tularensis cell viability to the altered permeability resulting from aerosolization and rehydration.

Hatch and Dimmick (57) related the death rate of microorganisms aerosolized at various relative humidities to an inbalance in metabolic function prior to aerosolization.

Greene (79) exposed aerosols of lyophilized Serratia marcescens and Bacillus subtilis var. niger spores in a wind tunnel to temperatures ranging from 75-200 C for about one sec. S. marcescens required exposures of 150 C for 1.68 sec to 170 C for 0.5 sec for 99.9 percent kill. Exposures of 160 C for 1.68 sec to 200 C for 0.5 sec were necessary for 50 percent inactivation of B. subtilis var. niger spores. Protective effects were noted when the lyophilized suspensions were coated with colloidal silica powder before aerosolization.